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Neuronal Nitric Oxide Synthase (*NOS1*) Polymorphisms Interact with Financial Hardship to Affect Depression Risk

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There is increasing evidence that genetic factors have a role in differential susceptibility to depression in response to severe or chronic adversity. Studies in animals suggest that nitric oxide (NO) signalling has a key role in depression-like behavioural responses to stress. This study investigated whether genetic variation in the brain-expressed nitric oxide synthase gene *NOS1* modifies the relationship between psychosocial stress and current depression score. We recruited a population sample of 1222 individuals who provided DNA and questionnaire data on symptoms and stress. Scores on the List of Life-Threatening Experiences (LTE) questionnaire for the last year and self-rated current financial hardship were used as measures of recent/ongoing psychosocial stress. Twenty SNPs were genotyped. Significant associations between eight *NOS1* SNPs, comprising two regional haplotypes, and current depression score were identified that survived correction for multiple testing when current financial hardship was used as the interaction term. A smaller three-SNP haplotypes (rs10507279, rs1004356 and rs3782218) located in a regulatory region of *NOS1* showed one of the strongest effects, with the A-C-T haplotype associating with higher depression scores at low adversity levels but lower depression scores at higher adversity levels ($p = 2.3E-05$). These results suggest that *NOS1* SNPs interact with exposure to economic and psychosocial stressors to alter individual's susceptibility to depression.

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INTRODUCTION

Depression is one of the most common forms of mental illness and even mild forms can impact adversely on quality of life, in addition to extracting a wider financial toll in terms of lost man-hours and treatment expenditure. A 2007 survey of adult psychiatric morbidity (McManus *et al*, 2009) estimated that 17.6% of adults in England have some forms of common mental disorder (CMD), with over half (9% of total) of the respondents with CMDs presenting with mixed anxiety and depressive disorder, which at the time was estimated to account for 20% of lost working days (Das-Munshi *et al*, 2008). This report also highlighted a link between financial hardship and depressive episodes, with depression rates rising from 0.2% in men in the highest adjusted income quartile to 6.9% in the lowest. A number of studies that have suggested that recent or current financial hardship has a role in the initiation or maintenance of depressive episodes, over and above the effects of other

measures of socioeconomic position and demographic characteristics, which are known to be associated with the development of depression and related disorders (Butterworth *et al*, 2009, Meltzer *et al*, 2010, Hosang *et al*, 2012). In addition, unlike many other psychosocial stressors the effect appears to be consistent across both sexes (Maciejewski *et al*, 2001). This may be because financial hardship is acting as a proxy for other forms of deprivation and social stress, linking it more closely to personal survival than other measures of stress. There is also evidence that early socioeconomic stress is associated with epigenetic changes in the serotonin transporter (Beach *et al*, 2014), supporting the idea that genetics, epigenetics, and past environmental exposures interact to alter our susceptibility to environmental influences.

The nitric oxide (NO) pathway has a major role in neuronal signaling. In humans NO is produced by three nitric oxide synthases (*NOS*). The most abundant form in the brain is neuronal NOS (*NOS1*), which has a broad role in synaptic signaling as well as being implicated in learning, memory, neuronal plasticity and a number of psychiatric disorders, including depression (Steinert *et al*, 2010, Doucet *et al*, 2012), and schizophrenia (Weber *et al*, 2013). More specifically, *NOS1* has a role in the regulation of the serotonin pathway (Pogun and Kuhar, 1994) and the HPA axis (Zhou *et al*, 2011) and is coupled to Ca^{2+}-permeable N-methyl-D-aspartate (NMDA)-receptors (NMDAR) at the

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postsynaptic density (PSD-95), through their mutual PDZ-binding motifs (Weber *et al*, 2013). It is also stress-responsive, with chronic stress increasing NOS-I expression in many parts of the brain, including the hippocampus in animal models (Joca *et al*, 2007, Zhou *et al*, 2011). A number of animal experiments report that NOS-inhibiting drugs exert antidepressant-like behavioural effects in stress models (Silva *et al*, 2012).

SNPs in the *NOS1* region (12q24) have been associated with major depression (McGuffin *et al*, 2005, Galecki *et al*, 2011) and with general psychological distress in a large multicentre GWA meta-analysis (Luciano *et al*, 2012). The present study tested the prediction that the influence of genetic variation in the *NOS1* gene on current depression would be more apparent when tested in relation to recent/current adversity rather than in isolation. It also compared results obtained using a simple five-point self-report current financial hardship scale as a measure of current psychosocial stress to those obtained using recent (within the last year) List of Life-Threatening Experiences (LTE) scores, as the LTE has been shown to have a high test–retest reliability and good correlation with more detailed semistructured life event interviews (Brugha and Cragg, 1990). The study cohort is based on a population sample recruited for the NewMood (New Molecules in mood disorders) study, which was designed to identify genes associated with depression and other mood disorders (Juhasz *et al*, 2009).

MATERIALS AND METHODS

Study Cohort

The study was approved by the local Ethics Committee and was carried out in accordance with the Declaration of Helsinki. All participants provided written informed consent. Subjects aged 18–60 years were recruited, predominantly from Greater Manchester, United Kingdom, as a population sample through general practices and a website (http://www.newmood.co.uk). Full details of the recruitment strategy, data collection, including how all the measures were derived and validated can be found in the Supplementary Information and has been published previously (Juhasz *et al*, 2009).

A questionnaire pack and genetic sampling kit were sent to the participants by post. The questionnaire contained a background questionnaire (Juhasz *et al*, 2009), which included demographic, health and lifestyle measures, and an inventory of the individual's personal psychiatric history. Depression within the last week was measured using the depression subscale of the self-report 53-item Brief Symptom Inventory (Derogatis, 1993).

Current financial hardship was assessed using a self-report five-point scale (1 = living very comfortably, 2 = living quite comfortably, 3 = just getting by, 4 = finding it difficult to make ends meet, 5 = not able to make ends meet). The List of LTE within the last year was used to measure recent negative life events (Brugha and Cragg, 1990). In the *post hoc* analysis four subsets of the LTE were considered, consisting of questions relating to intimate relationships (Category 1; Q5 and 6), finance and employment (Category 2; Q8–10), illness/injury/problems (Category 3; Q1, 7, 11, and 12),

social network, including loss of partner (Category 4; Q2–4). Full details can be found in Brugha *et al* (1985) and Rijsdijk *et al* (2001).

Individuals who reported manic or hypomanic episodes, psychotic symptoms, or obsessive-compulsive disorder were excluded and the experimental cohort was limited to individuals of self-reported Caucasian ancestry as this was the largest ethnic group, producing a cohort of 1222 (Supplementary Table S1).

SNP Selection and Genotyping

Genomic DNA was extracted using the Freeman *et al* (2003) protocol from buccal mucosa cells collected by cytology brush (Cytobrush plus C0012; Durbin PLC). The SNPs (Table 1) were selected based on the International HapMap Project Phase I. June 2005 release (http://hapmap.ncbi.nlm.nih.gov/, CEU population) using the HaploView software (http://www.broad.mit.edu/personal/jcbarret/haploview/). The 20 selected SNPs were estimated to impute ($r^2 > 0.8$) 37% of the SNPs in the gene region (65-kb downstream and 5-kb upstream of gene to include potential regulatory regions) using an updated HapMap release (hapmap_rel27), with the majority of the coverage focused on the exons and regulatory regions. Genotyping was carried out using the IplexTM assay from Sequenom MassARRAY technology (Sequenom, San Diego) following the manufacturer's protocol (http://www.sequenom.com). Genotyping was carried out blind to phenotype. A 15% replication of genotyping was built into the study design, from which an overall type I error rate of 0.016% was calculated.

Statistical Analysis

Statistical analysis was carried out using R Commander (Fox, 2005). Owing to the sometimes modest number of minor homozygotes all primary analyses were carried out using both additive (Add) and dominant (Dom; minor homozygotes grouped with the heterozygotes) genetic models. A false discovery rate (FDR; Benjamini and Hochberg, 1995) of 5% (*Q*-value: http://genomics. princeton.edu/storeylab/qvalue/) was used to produce *p*-values corrected for multiple testing. As LTE and financial hardship were to be compared as measures of current psychosocial stress, the models containing them were corrected separately.

Significant skewing of the distribution of potentially confounding factors between genotypic groups was checked for in sex, age, marital status, employment status, education, housing situation, long-term health issues, current general health, financial hardship, LTE, social support problems, and childhood adversity.

The statistical analysis looked at the effect of genotype stress interaction on depressive symptom score. In all analysis current depression score was used as the outcome measure and the analysis was performed separately for LTE score (within the last year) and the five-point current financial hardship. As would be expected in a population sample, the BSI depression score has a non-normal distribution with 19% of participants reporting no depressive symptoms (Supplementary Information), a Poisson regression model was used with age and sex included as covariates.

Table 1 SNP Location, HWE, and Frequency Information

SNP ID	SNP	Postion	Function	Alleles	Genotype distribution (N)			MAF	Missing genotype	HWE
					Major	Het	Minor			
1	rs41279104	117877485	Promoter	G/A	825	183	12	10.2%	16.7%	0.61
2	rs4767533	117793530	Intron	C/T	699	410	84	24.2%	2.5%	0.03
3	rs693534	117784718	Intron	G/A	489	561	151	35.9%	1.8%	0.63
4	rs527590	117781918	Intron	C/T	728	344	57	20.3%	7.7%	0.05
5	rs10507279[a]	117780274	Intron	G/A	836	352	32	17.0%	0.5%	0.39
6	rs1004356[a]	117777372	Intron	T/C	796	377	39	18.8%	1.0%	0.48
7	rs579604[b]	117773184	Intron	C/T	811	362	41	18.3%	0.8%	0.92
8	rs3782218[a]	117771511	Intron	C/T	771	332	38	17.8%	6.7%	0.86
9	rs816296[b]	117770744	Intron	C/A	807	355	39	18.0%	1.8%	0.99
10	rs9658281	117767578	Intron	G/A	570	512	118	31.2%	2.0%	0.87
11	rs1520810[b]	117765189	Intron	A/T	808	361	39	18.1%	1.2%	0.76
12	rs561712	117752069	Intron	G/A	477	538	153	36.2%	4.5%	0.96
13	rs522910	117736905	Intron	C/T	313	586	313	49.3%	2.4%	0.51
14	rs744664	117732101	Intron	G/A	1197	11	1	0.5%	1.3%	0.87
15	rs3782202	117720380	Intron	A/G	731	326	54	19.5%	9.2%	0.03
16	rs2293050	117718822	Intron	G/A	380	596	240	44.3%	0.7%	0.81
17	rs816357	117682766	Intron	G/C	778	150	11	9.1%	23.2%	0.36
18	rs7959232	117676698	Intron	A/G	514	554	132	34.1%	2.0%	0.33
19	rs1875140	117672339	Intron	A/G	780	379	63	20.7%	0.2%	0.06
20	rs3741477	117672233	Intron	T/C	1145	2	1	0.2%	6.3%	<0.0001

Abbreviations: HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency.
[a,b]Alleles (major/minor) a and b indicate two separate sets of SNPs in strong linkage disequilibrium ($r^2 > 0.8$). NOS1 is located at 12q24.22, SNP position is taken from dbSNP build 138.

Haplotypes were created using JMP Genomic v5's (Cary, NC, USA) Haplotype Estimation tool. Individuals with a final expectation maximization probability of less than 0.8 were excluded and haplotypes with frequencies of less than 5% were grouped. Additional linkage disequilibrium (LD) analysis was carried out using Haploview v4.2 (Broad Institute, Cambridge, MA, USA). In addition to haplotypic trend regression gene × environment interaction analyses, as described above, were repeated for each haplotype separately.

The function/potential function and evolutionary conservation of regions showing associations to the outcome measure was examined using ENCODE (Encyclopedia of DNA Elements) project data via the UCSC genome browser (http://genome.ucsc.edu/ENCODE/) and the evolutionary conserved region (ECR) browser (http://ecrbrowser.dcode.org/).

Additional *post hoc* analysis was carried out to check for sex-based biases and to examine the impact of specific LTE question subsets, including comparing individual responses to the five-point financial hardship scale and the LTE question 'Have you suffered from a major financial crisis?'

RESULTS

Demographic and Baseline Analysis

Analysis of each SNP's properties resulted in the exclusion of the *NOS1* SNPs rs3741477 and rs744664 from further analysis because of their low MAF within this study cohort ($<1\%$). In addition two SNPs, rs41279104 and rs816357, had over a 10% genotyping failure rate and were excluded from the haplotypic analysis. Three SNPs showed moderated but significant variation from Hardy–Weinberg equilibrium (Table 1).

No associations between genotype and demographic or lifestyle measures were identified, which passed an uncorrected significance threshold of $p = 0.01$, except for an association between rs9658281 and LTE ($p = 0.004$). LD analysis (Figure 1) identified two sets of three SNPs in strong LD ($r^2 > 0.8$; Haplotype A (HapA): rs10507279–rs1004356–rs3782218; Haplotype B (HapB): rs579604–rs816296–rs1520810). Supplementary Figure 1 shows regional D'.

Single SNP Analysis

Significant associations between eight *NOS1* SNPs and current depression (Table 2), with financial hardship as an interaction term, were identified that survived correction for multiple testing, using both additive and dominant models (rs693534, rs10507279, rs1004356, rs3782218, rs9658281, rs561712, rs522910, and rs2293050). Three of these SNPs (rs693534, rs561712, and rs2293050) also showed weaker but significant associations with LTE score as the interaction term. No significant associations were identified when financial hardship or LTE score were not included as an interaction terms.
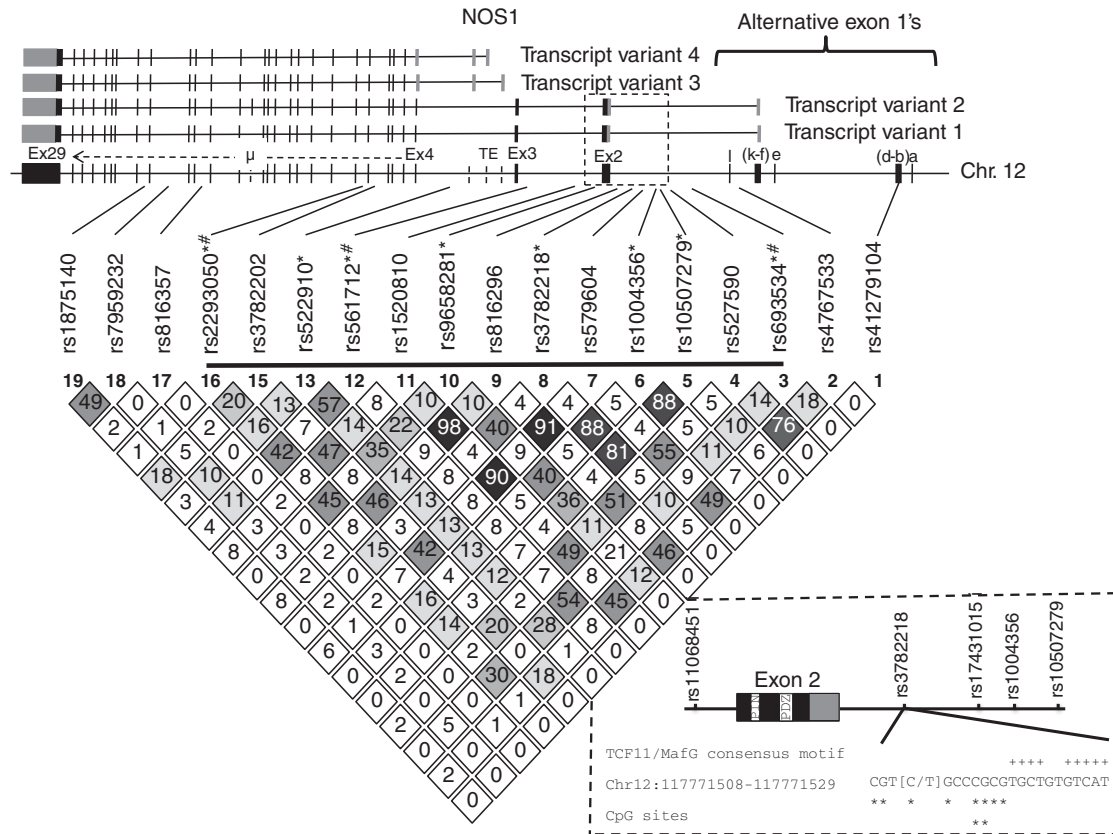
Figure 1 Schematic of the pairwise LD (r^2) for this study cohort, with extremely low MAF SNPs (<1%) excluded. The position of the SNPs is also shown in relationship to common NOS1 transcripts. Both NOS1 show complex tissue-specific regulation. NOS1 has 12 alternative exon 1's (a-l) with variant 1 (nNOSα; NM_000620.4) and variant 2 (nNOSμ; NM_001204218.1) starting at exon 1f, and variant 3 (nNOSβ; NM_001204214.1) and 4 (nNOSγ; NM_001204213.1) initiating at alternative start points in intron 3 (Bros et al, 2006, Steinert et al, 2010). The inserted schematic shows in greater detail the HapA SNPs in relationship to selected regulatory and function elements in exon 2 and part of intron 1. The inserted schematic is not to scale. rs561712 is located close to exon 3 and near proposed testis-specific exons (TE; Wang et al, 1997). Supplementary Figure 1 shows D'. Main key: Black, coding region; grey, untranslated regions (UTR); — an alternative tissue-specific exon; solid black line indicates those SNPs included in the regional haplotype; *significant with financial hardship as the interaction term; #significant with LTE as the interaction term. Insert key: PIN, site of action for the protein inhibitor of NOS1 (PIN); PDZ, the PDZ-binding motif allows NOS1 to bind to different proteins and the plasma membrane; i, indicates the imputed SNPs imputed by HapA SNPs in the HapMap CEU cohort; +, the consensus sequence for the TCF11/MafG transcription motif; *,potential CpG methylation sites; **, proven methylation sites (UCSF Brain DNA Methylation; www.genome.ucsc.edu).

Post hoc analyses using subsections of the LTE score showed that question 10 'Have you suffered from a major financial crisis?' produced the same pattern of significance as the 1–5 self-rated financial hardship scale. Analysis of the four LTE subcategories (Brugha et al, 1985, Rijsdijk et al, 2001) identified moderate associations for category 3 (Table 2), which covers serious personal illness/injury and legal or personal problems. No associations with intimate relationships (Category 1), finances/employment (Category 2), and social network, including loss of partner (Category 4), were identified (Results not shown).

Haplotype Analysis

To test the independence of the single SNP associations, haplotypes were created for the 66-kb region (13 SNPs; rs693534–rs2293050) containing the significant single SNPs from the primary current depression analysis. This produced five regional haplotypes with frequencies of greater than 5% (Figure 2), with the minor alleles of the significant SNPs associating with the two most common haplotypes

(Hap1 and Hap2), demonstrating that the single SNP findings are not independent of each other. Standard haplotypic regression analysis with age and sex as covariates and financial hardship or recent LTE score, as covariates rather than interaction terms produced no positive associations. However, when the full model was run for each haplotype individually using an additive model, Hap1 (p^{FDR} = 1.3E-04, risk) and Hap2 (p^{FDR} = 1.8E-03, protective) showed positive associations using financial hardship as the interaction term and Hap1 (p^{FDR} = 1.0E-04, risk) for recent LTE score (Supplementary Table S2).

A comparison of the rates of increase in depressive symptoms with increasing financial hardship for all haplotypes with an estimated frequency of over 5% showed that carriers of two copies of Hap1 had the steepest increase in depressive symptoms and carriers of one copy of Hap2 (p^{FDR} = 1.8E-03) and one copy of Hap1 showed the least increase (Supplementary Figure S2). Carriers of two copies of Hap2 showed a further decrease but only accounted for 1.8% of the cohort. Hap1 and Hap2 are complementary, with the exception of five SNPs (rs527590, rs3782202, and

Table 2 Single SNP Analysis of Current Depression Score

| Marker | Model | Genotype* financial hardship | | | Genotype* LTE score | | | Post hoc analysis of LTE sub-scores | | | |
| | | | | | | | | Genotype*Q10 | | Genotype*Cat 3 | |
		Z-value	p-value	p-value^FDR	Z-value	p-value	p-value^FDR	Z-value	p-value	Z-value	p-value
rs41279104	Add	0.80	4.2E-01	3.5E-01	0.97	3.3E-01	3.1E-01	−0.15	8.8E-01	0.46	6.5E-01
	Dom	1.20	2.3E-01	2.5E-01	1.31	1.9E-01	2.2E-01	0.07	9.5E-01	0.88	3.8E-01
rs4767533	Add	−0.91	3.7E-01	3.2E-01	−0.85	3.9E-01	3.3E-01	−1.20	2.3E-01	−0.72	4.7E-01
	Dom	−1.15	2.5E-01	2.5E-01	−0.48	6.3E-01	4.8E-01	−1.05	2.9E-01	−0.98	3.3E-01
rs693534	Add	3.67	2.4E-04	9.3E-04**	2.76	5.8E-03	1.2E-02*	3.04	2.4E-03**	3.07	2.1E-03**
	Dom	2.76	5.8E-03	1.2E-02*	3.01	2.6E-03	6.5E-03*	2.49	1.3E-02*	2.91	3.6E-03**
rs527590	Add	0.24	8.1E-01	5.4E-01	−1.31	1.9E-01	2.2E-01	−1.05	3.0E-01	−0.79	4.3E-01
	Dom	0.31	7.6E-01	5.3E-01	−0.90	3.7E-01	3.2E-01	−0.87	3.8E-01	−1.02	3.1E-01
rs10507279[a]	Add	−4.44	9.2E-06	1.6E-04**	−1.38	1.7E-01	2.2E-01	−2.24	2.5E-02*	−0.90	3.7E-01
	Dom	−4.32	1.5E-05	1.6E-04**	−1.34	1.8E-01	2.2E-01	−2.17	3.0E-02*	−0.55	5.8E-01
rs1004356[a]	Add	−4.28	1.9E-05	1.6E-04**	−1.02	3.1E-01	3.0E-01	−2.36	1.8E-02*	−0.57	5.7E-01
	Dom	−4.29	1.8E-05	1.6E-04**	−0.98	3.3E-01	3.1E-01	−2.50	1.2E-02*	−0.43	6.7E-01
rs579604[b]	Add	0.01	9.9E-01	5.9E-01	−0.24	8.1E-01	5.4E-01	−0.63	5.3E-01	−0.24	8.1E-01
	Dom	0.08	9.4E-01	5.9E-01	0.47	6.4E-01	4.8E-01	−0.21	8.3E-01	−0.15	8.8E-01
rs3782218[a]	Add	−4.04	5.5E-05	3.9E-04**	−1.56	1.2E-01	1.8E-01	−2.35	1.9E-02*	−0.70	4.9E-01
	Dom	−3.88	1.0E-04	6.1E-04**	−1.52	1.3E-01	1.9E-01	−2.61	9.1E-03*	−0.45	6.5E-01
rs816296[b]	Add	−0.27	7.9E-01	5.4E-01	−0.03	9.7E-01	5.9E-01	−0.55	5.8E-01	−0.05	9.6E-01
	Dom	−0.72	4.7E-01	3.7E-01	0.68	5.0E-01	3.9E-01	−0.17	8.7E-01	0.03	9.8E-01
rs9658281	Add	−3.36	7.9E-04	2.8E-03**	−1.75	8.0E-02	1.4E-01	−2.28	2.3E-02*	−2.81	5.0E-03**
	Dom	−3.70	2.2E-04	9.3E-04**	−1.25	2.1E-01	2.3E-01	−2.60	9.5E-03*	−2.26	2.4E-02*
rs1520810[b]	Add	−0.46	6.5E-01	4.8E-01	0.04	9.7E-01	5.9E-01	−0.58	5.6E-01	0.24	8.1E-01
	Dom	−0.91	3.7E-01	3.2E-01	0.72	4.7E-01	3.7E-01	−0.21	8.3E-01	0.39	7.0E-01
rs561712	Add	4.43	9.4E-06	1.6E-04**	3.14	1.7E-03	4.8E-03**	4.06	4.9E-05**	3.65	2.7E-04**
	Dom	3.11	1.9E-03	5.1E-03*	2.40	1.7E-02	3.3E-02*	3.29	1.0E-03**	3.64	2.7E-04**
rs522910	Add	−3.75	1.8E-04	9.3E-04**	−1.96	5.0E-02	9.2E-02	−3.37	7.4E-04**	−3.01	2.7E-03**
	Dom	−3.73	2.0E-04	9.3E-04**	−1.62	1.0E-01	1.6E-01	−3.09	2.0E-03**	−3.00	2.7E-03**
rs3782202	Add	−1.54	1.2E-01	1.8E-01	−0.41	6.9E-01	5.0E-01	−1.66	9.8E-02	−0.26	7.9E-01
	Dom	−1.38	1.7E-01	2.2E-01	0.38	7.0E-01	5.0E-01	−0.94	3.5E-01	−0.23	8.2E-01
rs2293050	Add	−3.25	1.2E-03	3.7E-03**	−2.77	5.6E-03	1.2E-02*	−2.51	1.2E-02*	−2.87	4.2E-03**
	Dom	−3.31	9.2E-04	3.0E-03**	−2.45	1.4E-02	2.8E-02*	−2.92	3.5E-03**	−2.32	2.1E-02*
rs816357	Add	−1.13	2.6E-01	2.6E-01	−0.04	9.6E-01	5.9E-01	−1.16	2.5E-01	0.23	8.2E-01
	Dom	−1.28	2.0E-01	2.2E-01	−0.01	9.9E-01	5.9E-01	−1.35	1.8E-01	0.43	6.7E-01
rs7959232	Add	−1.15	2.5E-01	2.5E-01	−0.76	4.5E-01	3.7E-01	−0.71	4.8E-01	−2.58	1.0E-02*
	Dom	−1.47	1.4E-01	2.0E-01	−1.32	1.9E-01	2.2E-01	−1.18	2.4E-01	−3.08	2.1E-03**
rs1875140	Add	−1.43	1.5E-01	2.1E-01	0.17	8.7E-01	5.6E-01	−0.80	4.3E-01	−1.32	1.9E-01
	Dom	−1.95	5.2E-02	9.2E-02	−0.20	8.4E-01	5.5E-01	−1.02	3.1E-01	−2.08	3.7E-02*

Abbreviations: Add, Additive model; Dom, Dominant model (minor homozygotes grouped with heterozygotes).
List of Life-Threatening Events (LTE) score question 10 reads 'Have you suffered from a major financial crisis?' LTE Category 3 covers illness, injury, and problems. Correction for multiple testing was performed using a 5% false discovery rate (FDR), post hoc comparisons are uncorrected. Increasing financial hardship uses a 1–5 rating scale. Current depression is measured using the brief symptom inventory (BSI) depression subscale
a and b indicate two separate sets of SNPs in strong linkage disequilibrium ($r^2 > 0.8$).
*p-value FDR between 0.05 and 0.005.
**p-value FDR < 0.005.

SNPs rs579604, rs816296, and rs1520810), which only discriminates for regional Hap4 (Figure 2).

These findings correlate well with the previous analyses as the minor rs561712 allele is predicted to tag for 78.8% of Hap1 and the minor alleles of rs10507279, rs1004356, and rs3782218 are similarly linked to Hap2 and these SNPs showed the strongest associations in the single SNP analysis (Table 2). As rs10507279, rs1004356, and rs3782218 are in
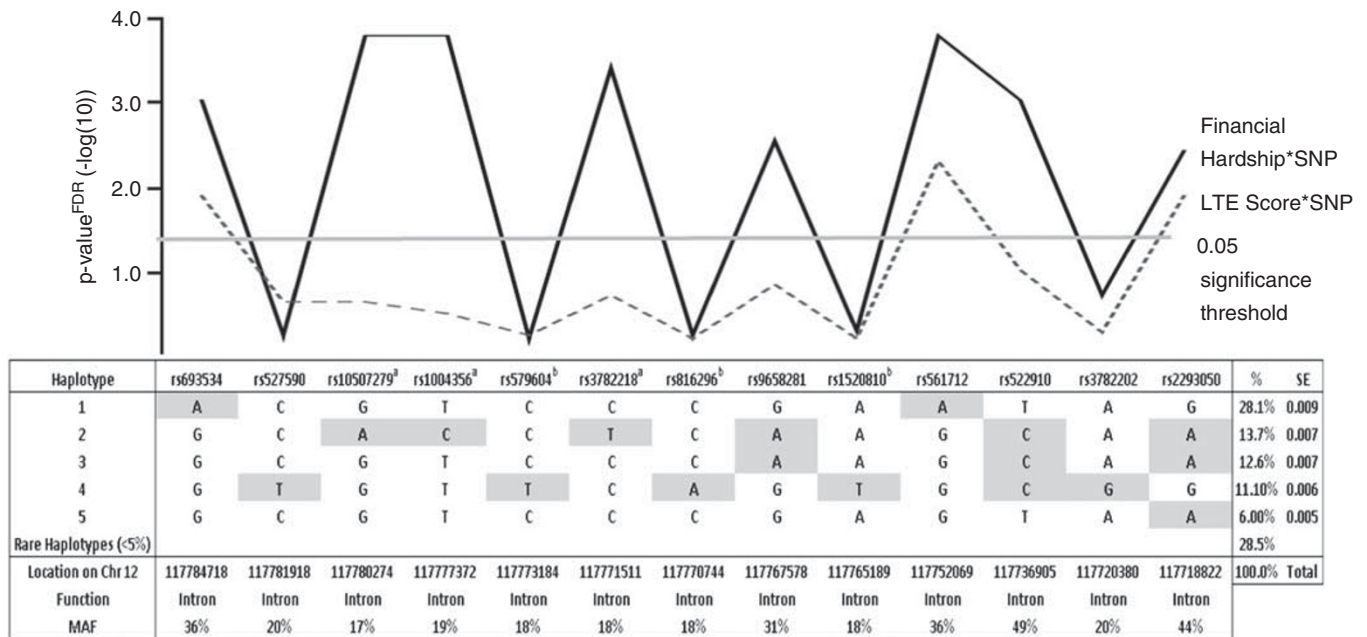
Figure 2 *NOS1* haplotypes and their relationship to single SNP findings. The haplotypes were created for the 13 SNPs located in the 66-kb region of NOS1, which contains all significant single SNP associations to current depression. Single SNP *p*-values corrected for multiple testing using a false discovery rate (FDR) of 5% are shown as –log10. Results for both primary models are shown. The trait variable, mean current depression score, was calculated using the brief symptoms inventory (BSI), and age and sex were included as covariates. Financial hardship or recent LTE score were included as an interaction term and main effect. MAF, minor allele frequency. SNP ID, rs numbers can be found in Table 1.
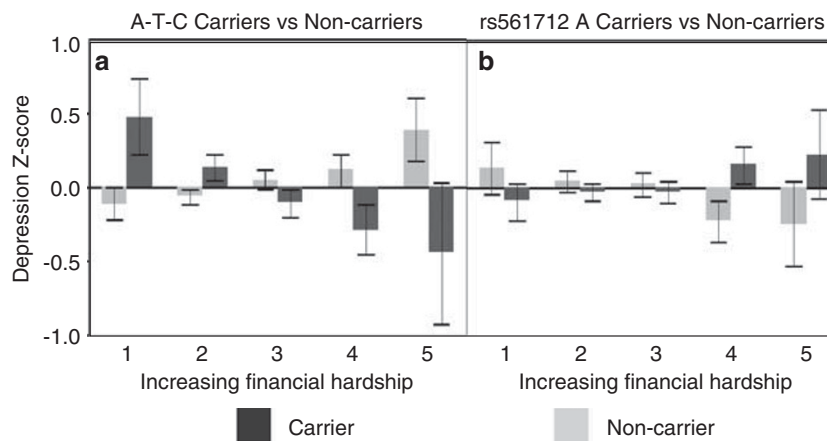
Figure 3 Effect of A-C-T and rs561712 minor A allele carrier status on variance from the mean for current depression score with increasing financial hardship. Both rs561712 A allele carriers and A-C-T carriers show an inversion of effect at financial hardship score 3, with the A-C-T carriers going having above mean to below mean and rs561712 A allele carriers showing the inverse. SE bars are included. The numbers in each subgroups according to financial instability level and haplotype/genotype combination are as follows: rs561712 A allele carriers: 1 = 60, 2 = 329, 3 = 216, 4 = 71, 5 = 13; rs561712 A allele non-carriers: 1 = 41, 2 = 291, 3 = 150, 4 = 53, 5 = 11; A-C-T carrier: 1 = 24, 2 = 154, 3 = 102, 4 = 32, 5 = 5; A-C-T non-carrier: 1 = 68, 2 = 356, 3 = 242, 4 = 84, 5 = 18.

near complete LD with each other, it is appropriate to consider them together and they will now be referred to as HapA (G-T-C = 80.4%, A-C-T = 16.2%, rare haplotypes = 3.4%). In 84.5% of cases, the minor A-C-T haplotype is associated with Hap2 and HapA showed a haplotypic (*p* = 2.3E-05, Dom and Add) association with current depression when financial hardship was considered, which was equivalent to the associations seen for the single SNPs (Table 2). This association reflects the fact that A-C-T

non-carriers had the smallest mean depression score at the lowest financial hardship score but showed a steeper increase in the mean depression score as financial hardship increased than A-C-T carriers (Figure 3a). This resulted in an apparent inversion of effect at financial hardship score 3, which corresponds to the answer 'just getting by'. This finding was mirrored by rs561712, with carriers of the minor A allele showing a more rapid increase in depression symptoms with increasing financial hardship (Figure 3b),

this could be explained by HapA and rs561712, respectively, tagging for Hap2 and Hap1, which are complimentary.

Sex Bias

In all analyses sex, which was included in the model as a fixed variable, was significant. No potentially confounding associations between sex and genotype were identified during baseline analysis. However, in this study the female proportion of the cohort had a significantly higher ($p < 0.0001$) mean current depression score than the male portion (mean ± SE; female, 1.2 ± 0.04 *vs* male, 0.8 ± 0.06). The current depression model with financial hardship as the interaction term was run for each sex separately (Supplementary Table S3) with females ($n = 849$) producing a similar pattern of significant association to the full cohort and males ($n = 373$) producing significant associations for the core of haplotype 2 SNPs (rs10507279, rs1004356, rs3782218, and rs9658281).

DISCUSSION

There is increasing evidence that genetic factors influence the long-term effects of early adversity (Buchmann *et al*, 2013, Heim and Binder, 2012) but less work has been undertaken on current stress in adults. This study builds on previous reports associating *NOS1* SNPs with current depression and psychological distress (McGuffin *et al*, 2005; Luciano *et al*, 2012) by demonstrating that the associations are likely to be mediated by responsivity to stress. The findings are compatible with findings in rodents implicating *NOS1* in behavioural, neural, and endocrine responses to stress (Zhou *et al*, 2011). In humans, post-mortem studies have shown altered *NOS1* levels in the anterior cingulate cortex (Gao *et al*, 2013) and hippocampus (Oliveira *et al* 2008) of depressed patients compared with non-depressed individuals, and the *NOS1* SNP rs6490121 has been associated with changes in prefrontal cortical function and grey matter density (Rose *et al*, 2012).

The strongest effects in this study were obtained for the simple five-point self-rated scale of current financial hardship, with eight SNPs and the two regional haplotypes they tag for producing associations, which survive correction for multiple testing. LTE question 10 'Have you suffered from a major financial crisis?' showed a similar interaction with *NOS1* genotype to the financial hardship scale but was less significantly associated to current depression (Table 2). This may reflect an advantage of the current financial hardship rating scale in measuring current financial stress as only 48% of those reporting that they were currently 'finding it difficult to make ends meet' ($n = 129$), and 76% of those reporting they were 'not able to make ends meet' ($n = 25$), considered that they had suffered from a 'financial crisis' in the last year.

Psychological stress is difficult to measure and there may be a number of explanations of why the findings for financial hardship are consistently higher than those for LTE, including that the financial hardship score is acting as a proxy for the deprivations and social difficulties that accompany financial hardship. Analysis of recent LTE score by subcategory only produced associations for Category 3

(Table 2), offering support to the idea that the type and timing of the psychological stress are important, as Category 3 covers serious personal illness/injury and legal or personal problems, which often have persistent or ongoing negative effects.

HapA and rs561712, which, respectively, tag for the regional haplotypes Hap2 and Hap1 the regional haplotype (Figure 2), both show what appears to be an inversion of effect at the level of current financial hardship score 3 ('just getting by'). Carriers of the minor A-C-T haplotype showed the most depressive symptoms when considering themselves financially stable, and the lowest depressive symptoms when they had moderate to severe financial problems (Figure 3a), whereas carriers of the minor rs561217 A allele showed the inverse pattern (Figure 3b).

Another inversion of effect in relation to stress has previously been observed for the functional *NOS1* promoter VNTR (ex1f-VNTR), which is located by exon 1f, in a study into impulsivity in Estonian adolescents. The authors suggest that in the presence of an adverse environment the impulsivity associated with the short form of the ex1f-VNTR switched from adaptive to maladaptive, thus reversing its potential selective advantage (Reif *et al*, 2011).

NOS1 expression is subject to complex regulation and produces a number of different isoforms, which differ in time course and according to tissue, and the type of stimulus. (Bros *et al*, 2006). This regulation includes regulatory sites in intron 1 and inhibitory elements within exon 2 (Jaffrey and Snyder, 1996) that are located firmly within the region of interest identified by this study (Figure 1 insert), suggesting that the observed inversion of effect could be because of a disruption in the regulation of the gene. Exon 2 also contains an *N*-terminal PDZ domain, which allows NOS1 isoforms containing exon 2 to bind to the plasma membrane and a number of different proteins (Bros *et al*, 2007). This includes the NMDA receptor, where it acts as a second messenger for the NMDA pathway (Weber *et al*, 2013). The NMDA pathway has repeatedly been associated with major depression (Mathews *et al*, 2012) and schizophrenia (Weber *et al*, 2013). In addition, the NMDAR/PSD-95/NOS1 complex has been a target for novel treatments of depression (Doucet *et al*, 2012).

Interestingly, the HapA SNP rs3782218, which is located 2-kb downstream from the start of exon 2, is located in an ECR, which suggests a regulatory function. It is also close to an ENCODE-confirmed lympho enhancer (Pu.1) motif and disrupts one of the CpG sites in a CpG cluster directly next to the consensus portion of a predicted binding motif for TCF11/MafG (Johnsen *et al*, 1996), a heterodimeric transcriptional repressor (Russo *et al*, 2004), further supporting a regulatory role for this SNP (Figure 1 insert). The same binding motif is present in the promoter region of *NOS2* and has been shown to repress the induction of *NOS2* expression in response to the cytokine TGF-beta as part of the inflammatory response (Berg *et al*, 2007).

In addition, selective methylation of CpG sites has a role in transcriptional regulation and the CGTCG motif, which is disrupted by rs3782218, is predicted to have a high probability of being involved in methylation-based regulation (Lu *et al*, 2010). Recent studies have reported gene-specific and global changes in DNA methylation in response to psychological stress in humans (Bick *et al*, 2012, Ursini

et al, 2011). These potential explanations could help to explain the inversion of effect seen for HapA, as a reduction or alteration in regulatory control may be detrimental under normal environmental conditions but beneficial under more stressful conditions. In addition, rs3782218 has previously been associated with early-onset Parkinson's disease (Hancock *et al*, 2008), which has stress as a risk factor and has been linked to excess NO (Aquilano *et al*, 2008).

The associations identified in this study are reasonably strong and are not only consistent with previous genetic and functional findings but also suggest a mechanism for them. Nevertheless, the study has several limitations. All ratings are based on self-completed questionnaires, and are therefore subjective and may be influenced by recollection bias. However, we have previously reported highly significant correlations between self-report and interview-assessed mood ratings in a subset of the participants using independent trained investigators in this cohort (Juhasz *et al*, 2009). As ethnic ancestry was not confirmed by either interview or targeted genotyping, undetected population stratification may be an issue but is likely to be limited as participants were recruited from a single suburban geographical location. In addition, there is always a degree of self-selection by participants based on motivation levels and interest in the subject matter in any form of population sampling; in this study this resulted in high levels of participation by individuals with a self-reported history of depression, many of whom were women; therefore, the study may not accurately represent the population it was recruited from. Additional information on these issues can be found in the Supplementary Materials and Methods.

Ideally, the results should be replicated in an independent sample; however, partial replication is afforded by the presence of the findings in separate analyses within each sex. Furthermore, the similarity of the interaction between genotype and both measures of recent adversity on current depression point to internal consistency. In addition, the low frequencies of some SNPs resulted in there being few carriers in the extreme adversity groups. Replication in a large independent cohort and functional analysis of rs3782218 would be advisable.

In conclusion, economic stressors are known to increase the risk of depressive episodes (McManus *et al*, 2009, Meltzer *et al*, 2010, Hosang *et al*, 2012) and this study may offer an insight into a potential biological mechanism for vulnerly to this type of stress. It also supports the 'differential susceptibility' hypothesis, which takes a more plastic approach to 'risk' alleles suggesting that under the right conditions these alleles may actually be beneficial (Belsky *et al*, 2009), such as in this case when the high-stress risk allele is associated with lower depression risk in the absence of financial hardship. Similar findings have been shown for the Serotonin transporter (5-HTT) with 5-HTTLPR short allele carriers showing higher depressive symptoms in the presence of stressful life events but lower depressive symptoms in their absence (Caspi *et al*, 2003, Belsky *et al*, 2009). Significant associations were limited to the highly regulated and functionally active region surrounding *NOS1*'s exon 2. The significant SNPs are in LD; therefore, it is difficult to suggest a mechanism by which these variants influence *NOS1*'s ability to modulate stress responsiveness; however, rs3782218 bears further investigation due to its potential regulatory role.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)